Bunn, S. E., and Green, A. J. A. (1983).—Oniscoidea (Crustacea: Isopoda) from Rottnest Island, Western Australia. *J. Roy. Soc. W. Aust.*, **65**: 147-151.

Ebert, T. A. (1982).—Longevity life history and relative body wall size in sea urchins. *Ecol. Monogr.*, **52**: 353-394.

Hodgkin, E. P. (1960).—Patterns of life on rocky shores. *J. Roy. Soc. W. Aust.*, **43**: 35-45.

Hutchins, B. (1979).—*A guide to the marine fishes of Rottnest Island.* Creative Research, Perth.

Johnson, M. S. and Black, R. (1982).—Chaotic genetic patchiness in an intertidal limpet. *Mar. Biol.*, **70**: 157-164.

Joll, L. M. (1975).—Reproduction and growth in two species of Turbinidae (Gastropoda: Prosobranchia). M.Sc. Thesis, University of Western Australia.

Joll, L. M. (1980).—Reproductive biology of two species of Turbinidae (Mollusca: Gastropoda). *Aust. J. Mar. Freshw. Res.*, **31**: 319-336.

Mahon, R. J. (1974).—A study of rock crabs of the genus *Leptograpsus* Milne-Edwards. Ph.D. Thesis, University of Western Australia.

Pearse, J. S. and Phillips, B. F. (1968).—Continuous reproduction in the Indo-Pacific sea urchin *Echinometra mathaei* at Rottnest Island, Western Australia. *Aust. J. Mar. Freshw. Res.*, **19**: 161-172.

Phillips, B. F. (1969).—The population ecology of the whelk *Dicathais aegrota* in Western Australia. *Aust. J. Mar. Freshw., Res.*, **20**: 225-265.

Phillips, B. F. and Campbell, N. A. (1968).—A new method of fitting the von Bertalanffy growth curve, using data on the whelk, *Dicathais. Growth* **32**: 317-329.

Phillips, B. F. and Campbell, N. A. (1970).—Comparison of methods of estimating population size, using data on the whelk, *Dicathais aegrota. J. Anim. Ecol.*, **39**: 753-759.

Phillips, B. F., Campbell, N. A. and Wilson, B. R. (1973).—A multivariate study of geographic variation in the whelk, *Dicathais. J. exp. Mar. Biol., Ecol.*, **11**: 27-69.

Shield, J. W. (1959).—Population studies in the littoral at Rottnest Island. In: Hodgkin, E. P. and Sheard, K. (eds.), *Rottnest Island: The Biological Station and Recent Scientific Research. J. Roy. Soc. W. Aust.*, **42**: 65-96.

Immunological studies on the quokka

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[This paper is dedicated to the late Harry Waring and our PhD students.]

Abstract

A major advantage of studying marsupial immunology is that the marsupial pouch-young, at birth, are at a much earlier stage of development than eutherian young. Much of the work cited in this review concerns the cellular and humoral responses of marsupials from which the cervical and thoracic thymus glands had been surgically removed shortly after birth. Specific emphasis on the quokkas developed as they are representative of a property unique to the Phalangeroidea—the possession of two sets of thymus glands. A concept of the immunological recovery following total thymectomy is presented and it is concluded that marsupial and eutherian immunology are essentially similar and that the mammalian immune system must have been fully developed prior to marsupial and eutherian divergence.

Introduction

"The immune system is a diffuse organ assigned to monitor the identity of the body. Its basic constituents are lymphocytes and antibody molecules, both of which recognise foreign molecules and one another."[1]

After 20 years' study, 40 publications and four doctoral theses, the only general statements I am prepared to make are:

1. marsupial and eutherian immunology are essentially similar in all major aspects; and
2. the mammalian immune system must have been fully developed prior to marsupial and eutherian divergence.

Peripheral to and supporting the generalizations is a large body of data comprising interesting technical and conceptual studies initiated 20 years ago when the discipline of immunology was at an early stage of development. Today we have developed techniques of exquisite specificity and sensitivity that reveal function and activity not possible to visualize when we started in 1963. Rather than disregard the sound observations of the past, it is more useful to recognise imaginative and skilful work as it comes into perspective.

This short review pays tribute to many contributors, particularly the late Harry Waring. As I recall, the initial stimulus came from Sir MacFarlane Burnet when he suggested that "Waring and Stanley do something about that quokka thymus". We did. The first presentation of our quokka immunology research was made in 1966 at an international CIBA Foundation Symposium on the "Thymus—experimental and clinical studies" in honour of Burnet and coinciding with the 50th anniversary of the founding of the Hall Institute of Medical Research (Stanley *et al.* 1966). Reproduced here are the original figures showing the development of the cervical and thoracic thymus glands and their location.

The potential of marsupials as experimental models for immunological function was not fully appreciated until Miller (1962) demonstrated the critical role of the thymus gland in maturation of some immune responses in the mouse. From this initial observation there have developed the classical studies on T and

[1] After Jerne, N. K. (1973).—*The Immune System*, pp. 49-57. Freeman, San Francisco.

B lymphocytes and a vast battery of tests involving cell function, immunoglobulin structure and function and ontogeny, evolution and genetics of the immune response. Without doubt, one of the major advantages of studying the immunology of marsupials is that their young are at a much earlier stage of development than eutherian young. This permits study of ontogenetic processes which may clarify aspects of physiological maturation which take place *in utero*, and thus are not easily accessible in eutherian mammals.

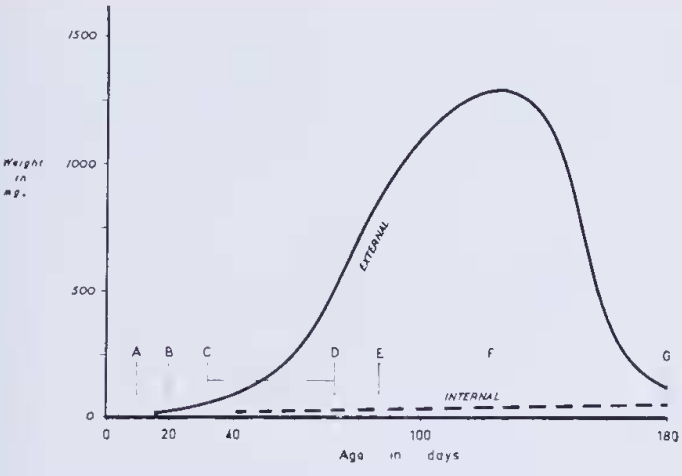


Figure 1.—Weight of internal and external thymus glands of quokka during pouch life.

A. Small lymphocytes apparent (both glands).

B. Cortex and medulla commence to differentiate (superficial).

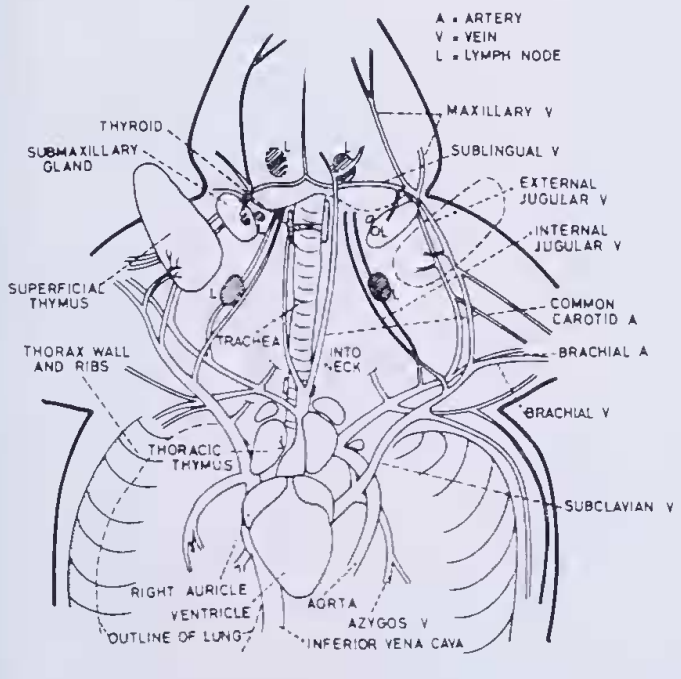
C and D. Densely compact small lymphocytes in cortex (both glands). Hassall's corpuscles located.

E. Large increase in lobules of external thymus.

F. Stands erect, underhair first apparent.

G. Joey leaves pouch.

With permission of the CIBA Foundation (see Stanley *et al.*, 1966, p. 209).



QUOKKA (150 DAYS OLD)

Figure 2.—Drawing of dissection to show location and blood supply of the superficial and thoracic thymus glands of the quokka.

With permission of the Australian Journal of Experimental Biology and Medical Science (see Yadav *et al.*, 1972a, p. 350).

For convenience the original work will be viewed from two separate but related areas (1) the thymus glands and cellular aspects, and (2) humoral (immunoglobulin) studies, followed by discussion of the findings in presenting the overall immunological picture—still far from being understood.

The thymus gland

Of 93 marsupial species examined by Yadav (1973), histologically-classical cervical thymus glands occur in only one superfamily, the Phalangeroidea. The question this observation poses is the evolutionary advantage that this extra thymus tissue confers—hence one of our initial interests was comparison of immune responses of *Setonix brachyurus*, which possesses both cervical and thoracic thymic tissue, with those of *Didelphis* marsupials which have only thoracic thymus tissue (Block 1964, Stanley *et al.* 1966, Yadav and Papadimitriou 1969, Ashman *et al.* 1971, Yadav *et al.* 1972 a and b, Stanley *et al.* 1972, Ashman *et al.* 1978).

Large lymphocytes appear in the cervical thymus of the quokka at 2-3 days and in the thoracic thymus at 5 days (Yadav *et al.* 1972 a, Lightowlers 1979). Much of the study of immune response consequently concerned the reactions of quokkas whose thymus glands had been removed surgically at different times and then tested for response to various immunological stimuli. Briefly the following categories of quokkas were studied in detail:

Intact and sham-thymectomised

Superficial thymus only removed
- before lymphocytes appear
- after lymphocytes appear

Superficial and thoracic thymus removed (Total thymectomy)
- before lymphocytes appear
- after lymphocytes appear

Table 1. shows the comparative development of lymphoid tissue and peripheral blood lymphocytes in *Setonix* and *Didelphis*.

The main antigens used in studying both marsupials were sheep red blood cells (SRBC), Φ X 174 bacteriophage, *Salmonella adelaide* flagellin, Bovine serum albumin (BSA), Dinitrophenyl-bovine serum albumin (DNP-BSA), rye grass pollen allergen (RPA), ovalbumin (OA), dinitro-phenyl-haemocyonin (DNP-Hc), and various mitogens and skin-grafts.

Although the cervical thymus becomes functional about 30 days before the thoracic thymus, there is no evidence to show that the glands have different functions (Stanley *et al.* 1972). A very large amount of the experimental surgical work involved "standard" total thymectomy—that is, cervical thymectomy before day 10 and thoracic thymectomy before day 20. This treatment delayed the first appearance of transplantation immunity until about 100 days of age. It was only in the latter years of our experiment, that it became technically possible to remove both sets of thymus glands prior to lymphocytopoiesis

Table 1

Comparative development of the lymphoid tissue and peripheral blood lymphocytes in the quokka (*Setonix brachyurus*) and the opossum (*Didelphis virginiana*). [Data from Block 1964, Yadav 1969, 1972, Ashman and Papadimitriou 1975].

Tissue	Lymphoid development	Age (days after birth) of first appearance of lymphoid development	
		Quokka	Opossum
Cervical thymus	Large and medium lymphocytes	1–2	
	Small lymphocytes . .	3	
	Hassall's corpuscles	14	
	Cortex/medulla differentiation	21	
Thoracic thymus	Large and medium lymphocytes	4	1
	Small lymphocytes	5	5–6
	Hassell's corpuscles	60	7
	Cortex/medulla differentiation	60	12
Lymph nodes .	Small lymphocytes	5	6
	Large lymphocytes	35	60–80
	Cortex/medulla differentiation	14	10–12
	Plasma cells	60–90	60–80
Spleen	Medium and small lymphocytes	7–14	17–20
	Large lymphocytes ...	7	65–100
Peyer's Patches	Medium and small lymphocytes	42	60
Blood	Medium lymphocytes	5	7
	Small lymphocytes ...	10	11

(Lightowlers 1979). These experiments enabled us to determine unequivocally whether the mammalian thymus is uniquely responsible for those immune responses classically described as thymus-dependent.

Prelymphocytopoieisis thymectomy did not affect growth, development and survival. Blood lymphocyte numbers were depressed and specific areas of lymph nodes and spleens showed marked lymphocytic depletion. E-rosette forming cells were absent for 130 days and SRBC antibody response not detectable until after 150 days. Juveniles, after leaving the mother's pouch developed these two responses and allogeneic skin grafts developed as rigorously as those of intact quokkas. In addition, the prelymphocytopoiesis-thymectomised juveniles responded to mitogenic stimulation by both phytohaemagglutinin (PHA) and concanavalin A (Con A).

It appears then that these responses, although delayed, are not uniquely dependent on thymus function in the quokka. There must therefore be an extra-thymic pathway for the development of the alleged classical thymus-dependent immunity. This could well be common to mammals.

Immunoglobulin studies

Following the initial responses of intact and thymectomised quokkas to SRBC, *S. adelaide* flagellin and x 174 hacteriophage antigens, the humoral responses were developed and extended by the staff of the Clinical Immunology Unit at the Princess Margaret Hospital under the direction of Kevin Turner. In a series of interesting papers, the following picture emerged. Unlike poikilothermic vertebrates which usually have only one class of immunoglobulin, the quokka has at least four immunoglobulin classes comparable to the IgG, IgM, IgA and IgE classes of eutherians.

The three antigens (SRBC, X 174, and flagellin originally used by Stanley *et al.*, (1972) to study the effects of thymectomy in the quokka were used by others to develop further the immunological picture. Yadav (1971) and Yadav and Eadie (1973) showed that maternal immunoglobulin (IgG) to these antigens appeared in the milk of immunized mothers and crossed the gut into the serum of pouch young. Bell *et al.* (1974) isolated IgG2, IgG1 and IgM in pure forms which differed in their electrophoretic mobility, molecular size, carbohydrate content and in the antigenic determinants of their heavy chains. Subclasses almost certainly exist with IgG2 and IgG1. This complexity is therefore comparable with eutherian mammals. Studies by Lynch and Turner (1974 a,b,c,) suggested an antigenic cross-reactivity hetween human IgE and the quokka homocytotropic antibody (HCA). This antibody elicited passive cutaneous anaphylactic reactions. If this is substantiated, it is further evidence suggesting that the immediate hypersensitivity system was developed prior to evolutionary divergence of marsupials and eutherians. However, the quokka HCA was indistinguishable from the IgG1 class which suggested to Turner and his colleagues that the extent IgG and IgE classes evolved from a primitive cytotropic chain. The IgA of the quokka has biological and physiochemical properties analogous to those of eutherian IgA (Bell *et al.* 1974).

Discussion

The thymus gland, cellular and humoral immune responses of the quokka so briefly outlined are only a small part of the complex immunological picture. Cockson and McNeice (1980) were aware of this when they reported on "survival in the pouch" and showed large numbers of alveolar macrophages present and both the macrophages and leucocytes in the milk filled stomachs of neonatal quokkas. Macrophages were also present in the milk and colostrum of lactating quokkas. These cells must surely be of significance to the immunologically incompetent embryo as it migrates from the birth canal along its path to the microbe-filled pouch in search of the teat. A study of the pouch microbial flora appears elsewhere (Charlick *et al.* 1981).

Much remains to be done with marsupials which are convenient models in immunological studies. In particular, studies with infective agents and carcinogens may well be productive, although we have not confirmed the observation of Jurgelski *et al.* (1976) who produced tumours in *Didelphis* with diethylnitrosourea (Stanley unpublished observations). The immunological recovery from neonatal thymectomy of the quokka has not been satisfactorily explained. Lightowlers (1979) suggested that the population of thymic-indcpendent lymphocytes associated with the return to immunological competence is the result of the appearance of "Q cells" from an extra-thymic pathway and that this is a phenomenon "common to mammalian immunology". On the basis of his E-rosette forming cells he suggests the foetal

liver be examined as a potential source. This speculation is interesting in the light of earlier comments by Ashman *et al.* (1978) on this particular problem when we stated "Although it is possible that another organ may take over the functions of the thymus in neonatally-thymectomized quokkas, it is difficult to see why this should function only early in life. Perhaps the thymus acts primarily as a biological amplification system, by providing a favourable environment for the proliferation of precursors migrating in from another source. In the absence of the thymus, these precursors may undergo spontaneous differentiation into clones of immunocompetent cells."

References

Ashman, R. B., Keast, D. and Waring, H. (1971).—The evaluation of the phytohaemagglutinin (PHA) response of peripheral leucocytes and skin graft rejection in thymectomized quokkas (*Setonix brachyurus*). *Proc. Aust. Soc. Med. Res.*, **2**: 454.

Ashman, R. B. and Papadimitriou, J. M. (1975b).—Development of lymphoid tissue in a marsupial, *Setonix brachyurus (quokka)*. *Acta Anat.*, **91**: 594-611.

Ashman, R. B., Stanley, N. F. and Waring, H. (1978).—Immunobiology of marsupials. In: *Animal Models of Comparative and Developmental Aspect of Immunity and Disease*, M. E. Gershwin and E. L. Cooper (eds.), 154-165, Pergamon Press, Oxford.

Bell, R. G., Lynch, N. R. and Turner, K. J. (1974).—Immunoglobulins of the marsupial *Setonix brachyurus* (quokka): characterization of three major serum classes immunoglobulin G-2, immunoglobulin G-1 and immunoglobulin M. *Immunology*, **27**: 1103-1115.

Block, M. (1964).—The blood forming tissues and blood of the newborn opossum (*Didelphys virginiana*). *Ergebrisse der Anatomie u Entwicklungsgeschichte*, **37**: 1-237.

Charlick, J., Manessis, C., Stanley, N. F., Waring, H. and Cockson, A. (1981).—Quantitative alterations of the aerobic bacterial flora of the pouch of *Setonix brachyurus* during oestrus, anoestrus, pregnancy and lactating anoestrus. *Aust. J. exp. Biol. med. Sci.*, **59**: 743-751.

Cockson, A. and McNeice, R. (1980).—Survival in the pouch: the role of macrophages and maternal milk cells. *Comp. Biochem. Physiol.*, **66A**: 221-225.

Jurgelski, W., Hudson, P. M. and Falk, H. L. (1976).—Embryonal neoplasm in the opossum; a new model for solid tumours of infancy and childhood. *Sci.*, **193**: 328-332.

Lightowlers, M. W. (1979).—Thymus function in the quokka: an analysis of mammalian thumus-dependent immunity. Ph.D. Thesis, Department of Zoology, University of W.A.

Lynch, N. R. and Turner, K. J. (1974a).—Time course of homocytotropic and passive haemagglutinating antibody synthesis in the marsupial *Setonix brachyurus*, the quokka. *Int. Archs Allergy appl. Immun.*, **47**: 933-941.

Lynch, N. R. and Turner, K. J. (1974b).—Immediate hypersensitivity responses in the marsupial *Setonix brachyurus* (the quokka). Biological properties of the homocytotropic antibody. *Aust. J. exp. Biol. med. Sci.*, **52**: 425-432.

Lynch, N. R. and Turner, K. J. (1974c).—Immediate hypersensitivity responses in the marsupial *Setonix brachyurus* (the quokka). Characterization of the homocytotropic antibody. *Aust. J. exp. Biol. med. Sci.*, **52**: 755-766.

Miller, J. F. A. P. (1962).—Immunological significance of the thymus of the adult mouse. Nature, **195**: 1318-1319.

Stanley, N. F., Waring, H., and Yadav, M. (1966).—The thymus: experimental and clinical studies. In: Ciba foundation Symposium, G. E. W. Wolstenholme and R. Porter (eds.), 207-213. Churchill, London.

Stanley, N. F., Yadav, M., Waring, H. and Eadie, M. (1972).—The effect of thymectomy on response to various antigens of a marsupial, *Setonix brachytrus* (quokka). *Aust. J. exp. Biol. med. Sci.*, **50**: 689-702.

Yadav, M. (1969).—The thymus glands of the (quokka) *Setonix brachyurus*, and their role in immunological processes. Ph.D Thesis, Department of Zoology, University of W.A.

Yadav, M. (1971).—The transmission of antihodies across the gut of pouch young marsupials. *Immunology*, **21**: 839-851.

Yadav, M. (1973).—The presence of the cervical and thoracic thymus lobes in marsupials. *Aust. J. Zool.*, **21**: 285-301.

Yadav, M. and Eadie, M. E. (1973).—Passage of maternal immunoglobulins to the pouch young of a marsupial, *Setonix brachyurus*. *Aust. J. Zool.*, **21**: 171-181.

Yadav, M. and Papadimitriou, J. M. (1969).—The ultrastructure of the neonatal thymus of a marsupial. *Setonix brachyurus*. *Aust. J. exp. Biol. med. Sci.*, **47**: 653-668.

Yadav, M., Stanley, N. F. and Waring, H. (1972a).—The thymus glands of a marsupial, *Setonix brachyurus* (quokka), and their role in immune responses. Structure and growth of the thymus glands. *Aust. J. exp. Biol. med. Sci.*, **50**: 347-356.

Yadav, M., Stanley, N. F. and Waring, H. (1972h).—The thymus glands of a marsupial, *Setonix brachyurus* (quokka), and their role in immune responses. Effects of thymectomy on somatic growth and blood leukocytes. *Aust. J. exp. Biol. med. Sci.*, **50**: 357-364.

Microbiology studies on Rottnest Island

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Abstract

Studies of four different groups of viruses, the bacterial flora of the pouch, and the ecology and pathogenesis of quokka filariasis, comprise the items of this review. The presence of reoviruses and arboviruses confirm the ubiquity of two major virus groups spread by entirely different means. The epidermal papillomata of the tails and feet of quokkas have been shown to be associated with a 'new' poxvirus. The Gram-negative bacterial flora of the pouch was dramatically reduced prior to birth. The life-cycle and immunobiology of quokka filariasis have been defined for the first time. Influenza A virus studies of birds have led to the isolation of a virus with a novel antigenic configuration.